OCT 1 1923

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XLV OCTOBER, 1923 NO. 4

CONTENTS

CHILD, C. M. *The Axial Gradients in Hydrozoa. V, Experimental Axial Transformations in Hydroids* 181

HARGITT, CHARLES W. *The Digestive System of the Periodical Cicada. II, Physiology of the Adult Insect* . . 200

HICKERNELL, L. M. *The Digestive System of the Periodical Cicada, Tibicen septendecim Linn. III, Morphology of the System in the Nymph* . . 213

PUBLISHED MONTHLY BY THE

MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY

LANCASTER PRESS, INC.

LANCASTER, PA.

AGENT FOR GREAT BRITAIN

WHELDON & WESLEY, LIMITED

2, 3 and 4 Arthur Street, New Oxford Street, London, W. P. 2

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Editorial Staff

E. G. CONKLIN—*Princeton University.*

GEORGE T. MOORE—*The Missouri Botanic Garden.*

T. H. MORGAN—*Columbia University.*

W. M. WHEELER—*Harvard University.*

E. B. WILSON—*Columbia University.*

Managing Editor

FRANK R. LILLIE—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, Prince and Lemon Streets, Lancaster, Pa.

Vol. XLV. *October, 1923.* *No. 4.*

BIOLOGICAL BULLETIN

THE AXIAL GRADIENTS IN HYDROZOA. V. EXPERIMENTAL AXIAL TRANSFORMATIONS IN HYDROIDS.

C. M. CHILD.[1]

The hydroid stolon usually appears in nature as a basal outgrowth readily distinguishable from the stem by its habit of growing in contact with surfaces rather than free in the water. Contact has often been regarded as a factor in determining its formation, but it has been observed by many investigators that in the reconstitution of isolated pieces of hydroid stems stolons may develop from that end of the piece which was originally apical, as well as from the basal end, even when these ends are not in contact with solid surfaces. In some cases also it has been observed that isolated pieces of certain species develop only stolons which may later give rise to hydranths or may continue to grow as stolons. Peebles (1900) states that this frequently occurs in pieces of *Hydractinea* and *Podocoryne* when they are left in dishes undisturbed without change of water. Loeb ('92) maintained that stolon formation in *Antennularia antennina* is determined by gravity, but Morgan ('01) and Stevens ('02, '10), while not denying the correctness of Loeb's conclusions, demonstrated beyond a doubt that other factors than gravity may be concerned. They were not able, however, to reach definite conclusions concerning the nature of these factors. Lund ('21) has shown that when isolated stem pieces of certain hydroid species are exposed to the electric current, stolons tend to arise at the end toward the cathode, hydranths at the end toward the anode. Various other data might be cited, but it seems fair to say that no one thus far has been able to discover a general physiological basis for the development of

[1] From the Hull Zoölogical Laboratory, University of Chicago.

stolons in hydroids. The original polarity, gravity, contact, the electric current, light, have all been mentioned as factors concerned in determining the polarity of hydroids, but no one of these has been shown to be of fundamental or general importance as regards stolon formation.

The present paper is concerned with observations and experimental data which throw some further light upon this problem. These data were accumulated during four summers, 1917–1920, spent at the Puget Sound Biological Station, and I take this opportunity of expressing again my obligation to the Director for the facilities afforded.

Material and Methods.

Three hydroid species, *Bougainvillea mertensi*, *Obelia borealis* and *Gonothyræa clarkii*[1] were chiefly used, but a few experiments were performed with other species.

In the course of other work with hydroids it was observed that often in *Bougainvillea*, and occasionally in other forms, freshly collected stocks showed stolons in place of hydranths. Commonly these stolons were found in the basal one fourth to one half of the stock and on the more basal secondary branches of the primary branches of this region. In some stocks this basal portion is a tangle of stolons with few or no hydranths, the stolons arising in part in the positions of hydranth buds and in part as apparently "adventitious" outgrowths.

A little later it was observed, particularly in *Bougainvillea*, that stocks which were entirely without these stolons when first collected often developed them after a few days in standing water in the laboratory. This suggested the possibility that such transformation of hydranth buds into stolons might be the result of depression or inhibition and experimentation with low concentrations of various inhibiting agents and conditions, such as crowding in standing water, keeping in closed dishes, etc., was begun.

In these experiments the following chemical agents and concentrations[2] were used: KNC $m/10000$, $m/25000$, $m/50000$, ethyl urethane, $m/200$, $m/500$; $MgSO_4$, $m/400$, $m/1000$; LiCl,

[1] I am indebted to Dr. C. C. Nutting for the identification of the *Obelia* and to Dr. Trevor Kinkaid for the identification of the *Bougainvillea* and *Gonothyræa*.

[2] Concentrations as given represent merely equivalents in sea water.

m/50; HCl, *m*/1500, *m*/5000; neutral red. With some concentration of each of these agents positive results were obtained, and crowding, keeping in closed dishes, and infrequent change of water also gave positive results.

In the experiments complexes, stocks or "colonies" lacking only the holdfast, or large complexes of stems and branches with a single cut surface at the basal end were used. These were placed in finger bowls holding about 400 c.c. of the experimental solution made up with well aërated sea water. When volatile agents were used the bowls were completely filled and covered with glass plates excluding all air, or all except a small bubble. Solutions were renewed daily or every two days, except in experiments to determine the effects of less frequent change. And finally, in some experiments the same concentration was continued throughout, in others the original concentration was replaced by a lower one, or the animals were returned to water after a day or two.

The figures are semi-diagrammatic but are all drawn from living specimens. Old stems, branches or thecæ which are empty because of disintegration or resorption of hydranths or retraction of cœnosarc are drawn in broken lines (Figs. 13–16, 18). Figs. 1–9, 11, 13 representing the development of stolons in earlier stages of experiment are drawn in outline without indication of cœnosarc, because all parts of stems and stolons contain it. In the other figures the cœnosarc is indicated by shading in order to show the later development and separation of stolons from the stock.

The chief purpose of the paper is the presentation of experimental data which show that transformation of stems and even of apical regions into stolons may occur under slightly inhibiting or depressing conditions. Questions of the range of effective concentrations, of regional, individual, specific and experimentally induced differences in susceptibility, and of the rate and degree of transformation are considered only incidentally or not at all.

Transformation in *Bougainvillea*.

Transformation of apical ends of branches into stolons often occurs in nature in *Bougainvillea*. In freshly collected stocks stolons are often found in place of some or all hydranths and

buds of the more basal regions, usually not more than the basal third or half, or as adventitious outgrowths from the branches of these regions. What factors are concerned in such cases is of course uncertain, but it may be pointed out that in these basal regions, which are often over-grown with plants and protozoa, less favorable conditions for respiratory exchange—retarded water movement, accumulation of CO_2, lack of oxygen—may directly inhibit and transform hydranth buds into stolons. On the other hand, if the whole stock is exposed to inhibitory conditions, the more basal regions, because of their lower rate of metabolism (Child, '19, '21; Hyman, '20), are in general less able to acclimate to such conditions than the more apical regions and may, therefore, undergo transformation, while other levels of the stock do not. Stocks which show no stolons at any level above the basal end when collected usually develop stolons over more or less of the stock after a day or two in standing water.

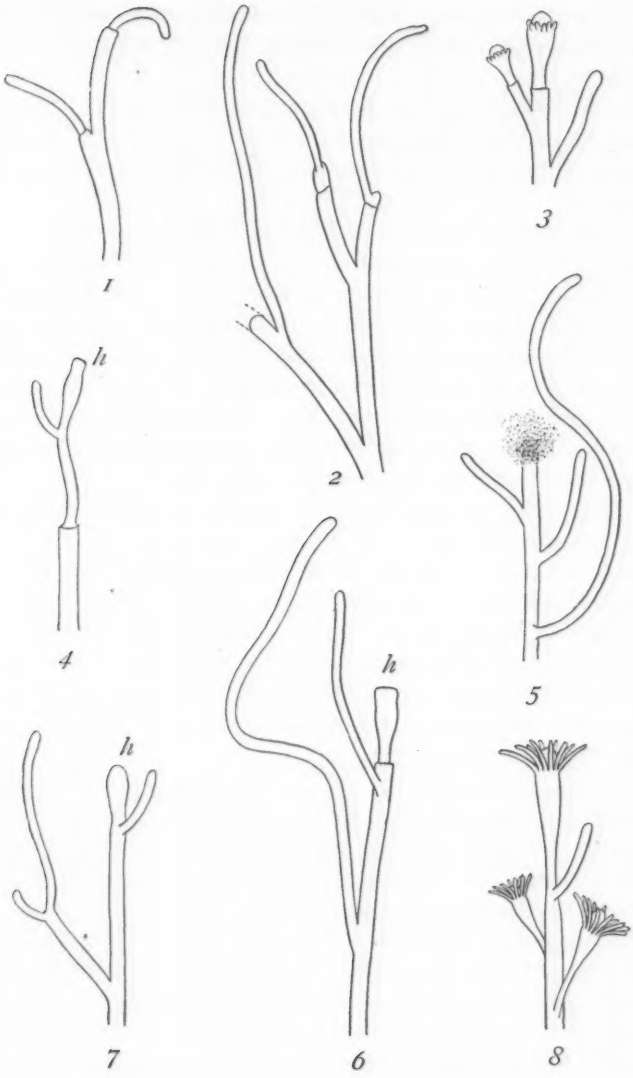
That such development of stolons from the more basal regions of stocks in water is not due to the presence or accumulation of "stolon-forming substances" in these regions or to any other specific factor is shown by the fact that this transformation can be induced in any or all regions of the stock, according to experimental conditions. The following experiments serve as examples:

1. Pieces of stocks in ethyl urethane, *m*/200. After forty-eight hours all hydranths disintegrated and numerous stolons developed, chiefly apical (Fig. 1) or subapical (Fig. 2).

2. Pieces in ethyl urethane for twenty-four hours, then returned to well aërated sea water. In ethyl urethane all original hydranths disintegrated and stolon development began. After twenty-four hours in water many subapical stolons were present, but new hydranths were developing and some stolons were transforming into hydranths and stems.

3. In ethyl urethane *m*/500 the original hydranths disintegrated and apical and subapical stolons developed within twenty-four hours, but after forty-eight hours many apical ends and in some cases the first subapical bud developed new hydranths and the stolons were inhibited (Fig. 3).

4. Pieces in ethyl urethane *m*/500 twenty-four hours, then returned to water, gave much the same results, except that in some cases the tips of the apical stolons themselves transformed


1
2
3
h
4
5
h
6
h
7
8

into hydranths after return to water (Fig. 4; *h*, hydranth bud).

5. In $MgSO_4$ *m*/400 almost all hydranths were disintegrated and subapical stolons grew rapidly within forty-eight hours (Fig. 5).

6. In $MgSO_4$ *m*/1000 disintegration of hydranths occurred and stolons developed much as in Expt. 5, but after forty-eight hours many new apical hydranths developed (Fig. 6; *h*, hydranth bud).

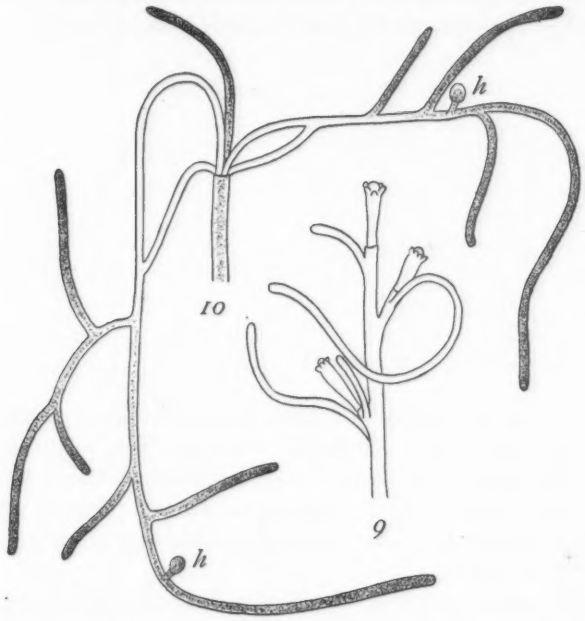
7. In sea water controls of Expts. 1–6 after forty-eight hours the original hydranths were more or less affected: tentacles were undergoing reduction or were completely gone and some hydranth bodies were disintegrated or disintegrating, or, in the case of the younger hydranths, resorbed. Subapical, or even apical stolons appeared on those axes in which the terminal hydranths were reduced or disintegrated (Fig. 7) and rarely short stolons appeared on stems with persistent hydranths (Fig. 8). On some stems new hydranths were developing Fig. 7, *h*).

8. In KNC *m*/10000 hydranths became motionless within a few hours and in the course of three or four days disintegrated, but in the course of six days no stolons developed. Solution was then changed to *m*/20000 and left exposed to air to permit slow decrease in concentration. A few short, subapical stolons developed during the next eight days, but no development of new hydranths occurred and medusa buds underwent resorption. Evidently development and growth were almost completely inhibited by this concentration.

9. In KNC *m*/25000 older hydranths disintegrated and younger underwent resorption. Stolons developed in subapical and lower regions. After six days solution was changed to *m*/50000 and left exposed to air. During next eight days an extensive stolon system developed from nearly all apical ends of all stems and branches and also from basal cut ends of pieces. No hydranths were present but a few hydranth buds appeared on the stolons. Results essentially like those of following experiment.

10. In KNC *m*/50000 the original hydranths disintegrated and stolons appeared and after six days small, partially inhibited new hydranths were present (Fig. 9). Solution was then changed to *m*/100000 in open dishes to permit gradual decrease in concentration. During the next eight days the new hydranths were

resorbed and extensive stolon systems developed from practically all tips (Fig. 10). These stolons bear a few erect stems with hydranth buds (Fig. 10; *h*, *h*), but these did not develop further.

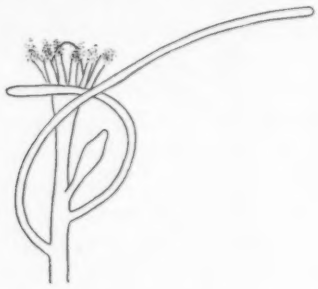


11. In HCl *m*/5000 ± pH 7.4, after forty-eight hours, most terminal hydranths of upper branches disintegrated and many subapical stolons present. Many hydranths at lower levels intact; no stolons. In controls in sea water, changed daily, terminal hydranths mostly disintegrated but new hydranths developing and very few stolons present.

12. In HCl *m*/1500 ± pH 6.9 hydranths disintegrate in one to two days, terminal hydranths usually first. After three days numerous stolons appeared in basal halves of pieces, but all development was inhibited in the apical halves. In sea water controls after three days the original hydranths were gone, many new hydranths were developing and few stolons appeared.

13. In pieces kept in standing water in closed vessels without air the changes during the first forty-eight hours are essentially

similar to those occurring in open dishes. More or less reduction and disintegration of hydranths and development of stolons occurs. Figure 11 shows the apical region of a stem in which the original terminal hydranth has reduced tentacles with disintegrating tips and the subapical hydranth bud has advanced but



11

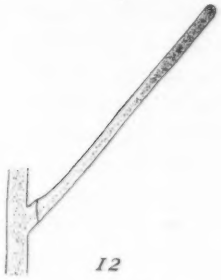
little during the forty-eight hours since collection. Below the bud two stolons have developed. In some other pieces in the same dish most or all hydranths appear intact, and no stolons or very few, chiefly in basal regions, are present. In still others apical hydranths are already completely disintegrated and stolons are growing rapidly.

In all cases in standing water the original hydranths disappear sooner or later and stolons develop, but a second and even a third generation of hydranths may arise. After a week or ten days, however, hydranths are usually entirely absent and all tips have either given rise to stolons or the cœnosarc is retracted. In short, the effect of standing water is essentiallly the same as that of the inhibiting agents, but less rapid. It is probably due in large part to accumulation of CO_2. There is always a decrease in pH in the dishes containing the pieces, often to 7.5 or 7.4 in the course of a week or less.

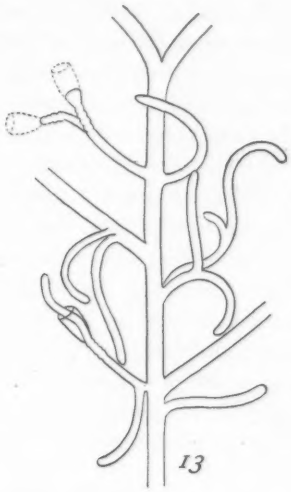
Transformation in *Gonothyræa*

Gonothyræa is much less susceptible to inhibiting agents and to standing water than *Bougainvillea*. The hydranths survive for several days or even a week under conditions which kill the hydranths of Bougainvillea in a day or two.

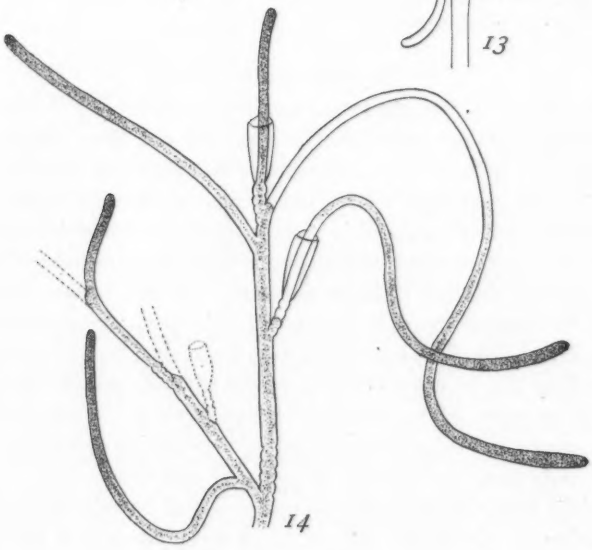
Some of the earlier experiments gave negative results as regards stolon formation, probably because the inhibiting conditions were not sufficient in degree or not continued long enough. Apparently however, the transformation occurs under less extreme conditions in stocks which have begun to produce gonozooids



12



13



14

than in those which have none, but my data indicate that transformation will occur to some extent in any stock with sufficiently high concentrations of the agent used and sufficient time. Lack of material limited experiment with this species. A few examples are given.

Pieces in HCl $m/2500 \pm$ (pH 7.3) still possess some hydranths after four days. A few short outgrowths, apparently intermediate between stems and stolons have developed but no typical stolons. These pieces were then changed to HCl $m/1500 \pm$ pH 6.8 and after two days more showed numerous stolons in the more basal regions and outgrowths apparently intermediate between stolons and stems in the apical regions. These intermediate outgrowths are straight, support themselves free in the water and do not adhere to surfaces as do the stolons, but they show no annulation and they develop quite independently of hydranth buds (Fig. 12).

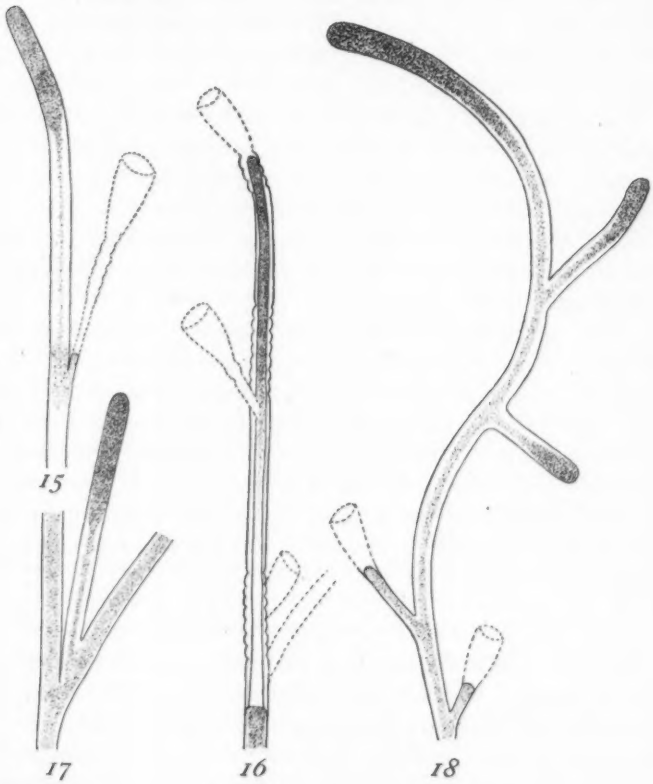
Fig. 13 shows the development of stolons in the basal region of a stock after a week in ethyl urethane $m/200$. In ethyl urethane $m/500$ transformation did not occur within a week. Figure 14 shows stolon development in the apical region of a stock after ten days in standing water. This stock bore numerous gonozooids in the basal regions.

Transformation in *Obelia*.

In *Obelia borealis* transformation was observed in HCl $m/1500$, LiCl $m/50$, chloretone $m/2000$, neutral red and also in standing water, but in water the stolons are less numerous than in the various solutions and after a few days new hydranths develop. In neutral red all parts stain opaque black, the hydranths disintegrate or are resorbed and stolons which are opaque black like other parts develop in large numbers. In this species stolons may be either terminal (Figs. 15, 16) or in the axils of branches (Fig. 17). Usually when they attain a certain length they separate from the parent stock, fall to the bottom and the tip continues to grow at the expense of lower levels until reduction to minute size and exhaustion occurs, or, if inhibiting conditions are removed, they may give rise to hydranth and stem.

Obelia geniculata behaves essentially like *O. borealis* as regards transformation and the stolons are of the same type in the two species. Another unidentified campanularian showed extensive

stolon formation after two days in standing water, many of the stolons being apical. Fig. 18 shows a case of apical transformation in this species.



Differences between Stolons and Stems.

There is usually no difficulty in distinguishing a stolon from a stem. The stem supports itself and is relatively rigid, the stolon is much less rigid and when it grows free in the water tends to hang downward as its length increases, until it comes into contact with a solid surface. Once in contact with a surface, the perisarc of the stolon adheres to it and its further growth is along the surface, but the stem does not attach itself to surfaces. It is not true, however, that contact is an essential factor in the origin

of stolons, but my observations suggest that in general stolons in contact grow to greater lengths and perhaps more rapidly than those free in the water. In the figures all the longer stolons are in contact with the glass of the container over most of their length (Figs. 2, 5, 10, 14, 18). The adventitious stolons in Figure 13 and the short stolons of Figures 1–5, 7, 8, etc., are not yet in contact with surfaces.

The stem grows only as part of a hydranth-stem complex or gradient, while the stolon may continue to elongate indefinitely without developing hydranths or hydranth buds. The direction of growth of stolons is apparently indefinite, or at least is readily altered, but the stem is usually straight. And finally, in those species in which annulations of the perisarc appear on the stems, the stolons are not annulated.

The "intermediate" outgrowths observed in *Gonothyræa* in HCl (p. 190 and Fig. 12) resemble stems in their rigidity and definite direction of growth, but, except in the development of the first hydroid from the planula, stems do not grow out in this manner without at least a hydranth bud at the tip. If the difference between stolon and stem is primarily one of "steepness" or other difference of the gradient, such intermediate outgrowths are possible and there is no good reason for doubting that the outgrowths observed are really intermediate.

The Process of Transformation.

The first step in the process is the inhibition or depression of the hydranths. The changes in the hydranths differ according to species and degree of depression, and range from decrease or cessation of motor activity for a day or two with subsequent recovery to complete disintegration or resorption. In *Bougainvillea* all the original hydranths usually disappear, even in standing water, within twenty-four to thirty-six hours and new hydranths may begin to develop within forty-eight hours. In *Gonothyræa*, on the other hand, the change from natural conditions to standing water has little effect on the hydranths; they remain intact for a week or more in most cases, but finally disintegrate or are resorbed as starvation advances.

The older, fully developed hydranths apparently always die and disintegrate, at least in large part, in all the species observed,

though it is possible that some portions may be resorbed, but younger developing hydranths or buds may be completely resorbed without visible loss of tissue. In *Bougainvillea* even the younger medusa buds underwent complete resorption under the more extreme inhibiting conditions, *e.g.*, KNC *m*/10000 six days, then gradual decrease in concentration. After two weeks the youngest medusa buds were completely resorbed, only the empty perisarc marking their position, somewhat older buds were partly resorbed and the most advanced buds had undergone disintegration.

The question of the nature of the process of resorption of hydranths was discussed some twenty years ago by Loeb ('00) and Thacher ('03), Loeb postulating a liquefying enzyme and Thacher interpreting the process as a degeneration. To all appearances the process is to some extent a real dedifferentiation. There is no visible loss of tissue and from what we now know of metabolic relations in the hydroids a simple interpretation of resorption and retraction of parts seems possible. The growing hydranths and medusa buds are regions of higher metabolic rate than the stems and are therefore able to appropriate the larger share of nutrition, but at the same time they are more susceptible to inhibiting or depressing conditions than stems (Child, '19, '21). When subjected to these conditions their metabolic rate decreases to a much greater degree than that of stems. Under such conditions they may not only be unable to maintain themselves, but their tissues, instead of taking nutriment from other parts, may become, whether through autolysis or other factors, a source of food for other parts. Consequently they may undergo decrease in size until nutritive equilibrium is established, or until the region with lower metabolic rate is more or less completely resorbed. Whether individual cells actually die in such a process or merely undergo dedifferentiation is difficult to determine, but in the light of what we know of the possibilities of dedifferentiation in these animals, it seems probable that cell death does not necessarily occur.

In starving hydroid stocks in the laboratory the retraction of cœnosarc in some regions and its outgrowth in others has often been noted. Tests of susceptibility and of permanganate reduction in such stocks indicate that the regions which are grow-

ing at any given time are regions of high rate of oxidation, while those which are undergoing retraction or resorption are regions of low rate. It seems probable then that in general the retraction of one part and the growth of another, particularly in starving stocks are associated with such differences in rate, the region of high rate maintaining itself and even growing at the expense of less active regions.

The normal relations and proportions of parts in nature must depend in large measure upon certain relations of rate of fundamental metabolism. When these metabolic relations are altered the form or proportions must change, and in simple, highly plastic forms such as hydroids, such changes may involve the complete resorption or atrophy of previously existing parts and the dominance and development of parts previously subordinate.

The stolon represents a physiological axis, a gradient (Child, '19, '21), but the data of susceptibility, $KMnO_4$ reduction and vital staining for some ten hydroid species examined indicate clearly that the stolon gradient is much less "steep" than the hydranth-stem gradient and that whenever, and wherever the gradient in a stolon becomes steep enough, transformation into a hydranth-stem complex occurs. In other words, the difference in rate between the hydranth bud or hydranth and the stem is greater than that between the stolon tip and the stem or the lower levels of the stolon.

According to this viewpoint, the stolon usually appears in nature as a basal structure, not because of the presence there of any "stolon-forming substances" but first, because this is the region of lowest metabolic or oxidative rate in the stock, and second, because new buds arising in this region are more or less inhibited by the dominance of the more active regions above. Probably the bioelectric currents resulting from the differences in electric potential between basal and higher levels are important factors in such inhibition. But whatever the factors involved, the partially inhibited axis develops in the form of a stolon. As I showed for *Tubularia* (Child, '15, pp. 91–2, 130–37), when the distance of the stolon tip from the hydranth becomes great enough, the stolon tip becomes physiologically isolated from the inhibiting action of the more active levels and transforms at once into hydranth and stem.

The experiments show, however, that stolon formation is not necessarily limited to basal regions of the stock, but may occur anywhere, even at apical ends, under inhibiting external conditions. In consequence of the differential susceptibility of different levels of the axis, the effect of such conditions is to decrease the steepness of the gradient. This change induces disintegration or resorption of hydranths, and new buds, instead of developing into hydranths, give rise to stolons. Moreover, a greater or less degree of physiological isolation of stem regions results from the disappearance of the dominant hydranths and in some species, as in many plants "adventitious" buds, *i.e.*, buds not localized in conformity to the usual order, arise (Figs. 11, 13). But under the inhibiting conditions the buds develop as stolons, not as hydranths. Such adventitious stolons have been seen most frequently in the basal halves or thirds of *Bougainvillea* and *Gonothyræa* stocks, but apparently may occur anywhere. And finally, the inhibiting conditions alter the steeper hydranth-stem gradients of terminal regions into the less steep stolon gradients and stolons therefore appear in place of hydranths.

Often more or less acclimation to the inhibiting conditions occurs in the course of a few days, and new hydranths begin to develop either from terminal regions which have not formed stolons (Fig. 7), from new buds, or by the transformation of stolon tips (Fig. 4). Such hydranth development retards or completely inhibits further growth of subterminal stolons near the hydranth, but the growth of terminal stolons may continue indefinitely (Figs. 10, 14, 15, 18) unless conditions are so altered as to induce their transformation into hydranth-stem gradients. In the case shown in Fig. 10, for example, each of the two chief stolon outgrowths gave rise at one point in its growth to a hydranth bud (*h*, *h*), but these two buds were unable to develop further than the stage shown in the figures and later underwent resorption.

Separation of Stolons from the Stock.

In all species investigated continued growth of the stolon leads sooner or later to loss of continuity between its cœnosarc and that of the parent stock. In *Bougainvillea* (Fig. 10) and *Gonothyraea* (Fig. 14) such separation of stolon and stock usually

occurs gradually and only after the stolon attains considerable length, but in *Obelia* it usually occurs at a relatively early stage and the region of separation is more sharply localized (Figs. 15, 17). Such differences, however, are not entirely constant for the species, for *Bougainvillea* stolons sometimes show a definite level of separation and *Obelia* stolons sometimes do not.

After separation of the cœnosarc the stolon may remain connected with the stock by the perisarc. In the case of stolons hanging free in the water the empty perisarc usually breaks and the stolon falls to the bottom, attaches itself and continues to grow, the tip growing at the expense of more basal levels until exhaustion occurs, or until conditions are altered so that the tip can transform into a hydranth and stem. Such free stolons may cover many centimeters of distance, leaving behind them a tube of empty perisarc as they go, and decreasing in length as their substance is gradually used as nutrition. In the laboratory this growth may continue for three weeks or even more, according to temperature, and while transformation into hydranths often occurs in the early stages, it apparently does not take place, even in favorable environment, in the later stages, but the stolon continues to "creep" over the bottom until reduced to a minute amount of cellular material. And even when growth ceases the small masses of tissue in the perisarc remain alive for some time longer.

The separation and continued growth of these stolons receives a simple physiological interpretation in terms of the axial gradient. If the stolon is such a gradient, the levels of relatively high rate are able to live to some extent at the expense of lower levels. Under laboratory conditions, without intake of food, the growth of the stolon tip is possible only at the expense of other parts. In the early stages the stolon tip, as a region of higher metabolic rate than the old stem cœnosarc, is able to take material from the latter, but as the stolon elongates the growth of the tip occurs more and more exclusively at the expense of the lower stolon levels, because the stolon gradient, and consequently the nutritive concentration gradient, is limited in length and when the length of the stolon exceeds this limit, it can no longer draw on the stock for nutrition.

From this stage on, the lower levels of the stolon gradient

gradually lose material to the higher levels and finally the cœnosarc of these levels disappears completely and separation of the stolon occurs. In Fig. 10 the two chief stolons have already separated from the original stock, and some of their longer branches are approaching separation from each other. In Fig. 14 also the two largest stolons are separated and a third is approaching separation. In Fig. 15 the terminal stolon of *Obelia* is almost separated, in Fig. 16 separation is complete, except as regards perisarc, and in Fig. 17 an earlier stage is shown.

After separation growth goes on as long as the regions of higher rate are able to take material from those of lower rate. In such stolons, even after a week or two of growth, the tip appears well fed and the cell layers are thick while toward the base the layers become progressively thinner and the cells more shrunken.

It is not yet known whether the rate of oxidation increases in advanced starvation in hydroids as it does in *Planaria* and various other animals, but apparently either this occurs at the lower levels more rapidly then at the upper levels of the separated stolon, or else the rate of oxidation in the upper levels decreases as the supply of nutritive material decreases. Either change leads gradually to the obliteration of the gradient, and as the cells become more and more alike in condition, growth becomes slower and slower and finally ceases.

According to this interpretation then the continued growth of such separated stolons in the absence of food from without is a simple physiological consequence of the fact that they represent physiological gradients and likewise the difference in appearance of the cœnosarc from the well filled tip to the shrunken, almost transparent base is another expression of the gradient. It may be suggested further that such stolons fail to develop hydranths in the more advanced stages of starvation because the gradient cannot attain the steepness necessary for hydranth formation. So far as I know, no other adequate physiological interpretation of these various facts has been advanced. The separation and continued growth of stolons may be an adaptation for purposes of reproduction under unfavorable conditions, but even if this is the case, the necessity for physiological interpretation still exists.

Conclusion and Summary.

It is evident that the formation of stolons in the hydroid species investigated is not dependent on region of stock, gravity, or contact, but rather on a certain degree of inhibition or depression, which may be determined either by relations to other parts of the stock or by external factors. Theories of physiological polarity based on distribution or direction of flow of hypothetical "formative stuffs" or upon molecular polarity and orientation afford no satisfactory interpretation of the facts presented in this paper. On the basis of such theories we must assume that placing the animals in standing water or in the experimental solutions must alter fundamentally the distribution or direction of flow of the formative stuffs, or must alter the molecular orientation or polarity in many different ways. But there is not the slightest reason for believing that such changes in conditions could accomplish any of these results. In terms of physiological gradients, however, all the facts are readily and simply accounted for and brought into line with other facts, and the earlier observations concerning such transformations and changes in polarity are likewise easily interpreted. In this field, as in many others the gradient conception affords a basis for the interpretation and synthesis of data which has previously been lacking.

In view of apparently persistent misunderstanding of the gradient conception, it is perhaps necessary to emphasize once more the fact that it is concerned with the physiology of development, not with heredity. In other words, the specific protoplasm of *Bougainvillea*, of *Gonothyræa*, or of *Obelia*, with all its hereditary potentialities, whatever these may be, is in each case the basis in which the gradient appears. This conception merely holds that, given this or any other specific protoplasm, the physiological gradient is an essential and fundamental factor in the realization of the hereditary potentialities in the form of an axiate individual of the species to which the protoplasm belongs.

The chief points are summarized as follows:

1. In various hydroid species the development of stolons can be induced by slightly inhibiting or depressing conditions, *e.g.*, low concentrations of ethyl urethane, $MgSO_4$, KNC, HCl, LiCl etc. and in most species even by change from natural conditions to standing water in the laboratory.

2. Stolon formation can be induced at all levels of the stock. The stolons may arise as adventitious outgrowths, by transformation of hydranth buds, or by transformation of terminal regions of stems after disintegration or resorption of hydranths.

3. These facts, together with data concerning the physiological gradients in hydroids indicate that the stolon axis is a somewhat inhibited gradient and iess "steep" than the hydranth-stem gradient. The separation of stolons from the stock and the continued growth of stolon tips at the expense of lower levels in the absence of food are regarded as necessary consequences of the presence in the stolon of an axial gradient.

REFERENCES.

Child, C. M.

'15 Individuality in Organisms. Chicago.

'19 The Axial Gradients in Hydrozoa. II. BIOL. BULL., XXXVII.

'21 The Axial Gradients in Hydrozoa. IV. BIOL. BULL., XLI.

Hyman, L. H.

'20 The Axial Gradients in Hydrozoa. III. BIOL. BULL., XXXVIII.

Loeb, J.

'92 Untersuchungen zur physiologischen Morphologie der Tiere. II. Würzburg.

'00 On the Transformation and Regeneration of Organs. Amer. Jour. Physiol., IV.

Lund, E. J.

'21 Control of Organic Polarity by the Electric Current. I. Jour. Exp. Zoöl., XXXIV.

Morgan, T. H.

'01 The Factors that Determine Regeneration in *Antennularia*, BIOL. BULL., II.

Peebles, Florence.

'00 Experiments in Regeneration and in Grafting of Hydrozoa. Arch. f. Entwickelungsmech., X.

'02 Further Experiments in Regeneration and Grafting of Hydroids. Arch. f. Entwickelungsmech., XIV.

Stevens, N. M.

'02 Regeneration in *Antennularia ramosa*. Arch. f. Entwickelungsmech., XV.

'10 Regeneration in *Antennularia*. Arch. f. Entwickelungsmech., XXX.

Thacher, H. F.

'03 Absorption of the Hydranth in Hydroid Polyps. BIOL. BULL., V.

THE DIGESTIVE SYSTEM OF THE PERIODICAL CICADA. II. PHYSIOLOGY OF THE ADULT INSECT.

CHARLES W. HARGITT.

The periodical cicada, now technically known as *Tibicen septendecim*, is well characterized by Marlatt (Bulletin No. 71, Bureau of Entomology, 1907, p. 11) as "undoubtedly the most anomalous and interesting of all the insects peculiar to the American continent. This cicada is especially remarkable in its adolescent period, the features of peculiar divergence from other insects being its long subterranean life of thirteen or seventeen years, during all of which time its existence is unsuspected and unindicated by any superficial sign, and the perfect regularity with which at the end of these periods every generation, though numbering millions of individuals, attains maturity at almost the same moment." Dealing with these several peculiarities and related problems of distribution, economic bearings, localized broods, etc., a large literature has accumulated since its first recorded advent at Plymouth, Mass., in 1623. But literature concerned with the more technical problems of its life history, for example, its embryology, morphology and physiology, is relatively small in volume and not of especially high value. Of some three hundred titles cited by Marlatt in the above-mentioned bulletin, by far the most of them relate to matters of habit, distribution, injuries caused, enemies, etc., and only an occasional reference to the anatomical or physiological problems concerned in its life. Much of this apparent indifference may be attributed to the highly obscure character of the life history of the insect, but not wholly so. For example, until the former paper by Hickernell appeared, there seems not to have been any critical account of the internal anatomy of any of the several organ systems of the insect. Nor has particular attention been directed to what must be rather unique physiological processes in such an organism. It is the purpose in what follows to submit an outline at least of the chief physiological observations which

have been made, extending over a series of years, with an effort to correlate them with the clearly established anatomical and morphological features already described. In this connection will also be reviewed some physiological results of recent times which seem to have intimate bearings upon the immediate problems before us. While there has been intimate and continuous coöperation and mutual aid between the writers of this paper, in almost all phases of the research, it is frankly stated, however, that each is independently responsible for his own contribution.

The critical interest of the senior author in the morphology and physiology of this insect began over twenty-three years ago at which time its emergence in June, 1899, afforded a novelty in laboratory material which was presented before a large class in zoölogy. While in the external morphology of the insect there was ready recognition of the general equivalents, or homology, already familiar from laboratory studies of crickets and grasshoppers, it was quite otherwise when dissection was undertaken and a study of the internal anatomy was attempted. Here it was soon apparent that conditions were so different from anything before studied as to be puzzling in the extreme, and it was decided that this part of the subject was beyond profitable attempt at anything more than a general, and rather superficial survey, especially as it came in the hurried closing days of the college year. At a later time this problem was assigned to a graduate student, R. L. Henderson, reference to which was made by the junior author in his previous paper. The generally accepted view among entomologists was that this insect seldom or never feeds during adult life, and in part this led likewise to the view that the digestive organs were more or less degenerate or even atrophied. Such was my own conclusion from the preliminary study above referred to, and presented in a paper read before the County Academy of Science in whose proceedings the latter appeared in print. When Henderson undertook the work assigned to him just cited above, he was early forced to discredit my earlier conclusions on this point; and while unfortunately he did not live to complete his research, some of that which he left in manuscript shows that he had obtained clear evidence of

the continuity and functional activity of the entire digestive system. The recent work by Hickernell has corrected and extended the investigation in a thorough and convincing way.

Feeding Habits.

A consideration of the feeding habits of cicadas is important in the present connection, as conditions in the digestive organs will be influenced by the reaction of the insect to food. As pointed out in the previous article, it has been maintained by various students of these insects that they seldom if ever take food during the adult life. Later studies by Quaintance, to which Marlatt has yielded a measure of assent, and the researches of the former paper already referred to, clearly establishing the functional efficiency of the digestive apparatus, renders the conclusion inevitable that there is no intrinsic difficulty in the views of these observers as to the feeding habits of the insect during the adult period of its life. The senior author has studied the food habits of this insect at several intervals for some twenty years and is not convinced that the accounts by Quaintance just mentioned are fully confirmed. Very special attention was given to this point during the appearance of the brood in this locality in the year 1916. In the main, it confirmed previous studies in 1899. In both cases attention was directed to two phases of the adult life, namely, that immediately following the emergence from the nymphal stage and during the early free-living condition, when it was believed the occasion to replace the wastes of this ordeal might express itself in free food taking. Furthermore, it was during this period of early adult life that accurate observations could be most easily made. But repeated observations at this time failed to show a single decisive case, though at times the attitude of the insect was such that it was necessary to disturb it in order to make certain that it was not actually feeding. Another period for observation was that about a week or ten days later when activity was very marked, especially among the males, in view of the growth of the reproductive organs and cells at this time. It was assumed that here was a special stage when need of food must be rather urgent. But here again, very few cases even under cage environment were distinguished. It must be admitted that during this period few specimens could

be found in which these observations were easily studied and, as before stated, it was found very difficult to distinguish between a merely resting or quiescent state and that of the attitude of the insect in feeding without disturbing its pose. To make this a matter of precision, numbers of insects were placed in breeding cages in the laboratory. These cages were provided with fresh stems of shrubs, and twigs from trees, and thus kept renewed daily for some time. At the suggestion of the senior author, an out-door insectary was constructed upon the lawn by using mosquito netting which was spread over growing shrubs and carefully fastened to the ground. In this enclosure, scores of specimens were kept under close observation for many days. But while cases of apparent feeding were now and then observed among these specimens, upon closer examination it was found that actually a very few were feeding, comprising less than 1 per cent of those under these conditions of observation. In view of these results, it has seemed to us that one could hardly accept the contention of Quaintance already cited that there was "frequent and general" feeding during the insect's life. The senior author's field observations on this matter have been rather extended. He has never observed what has been described as the abundant "exudation of greater or less quantity of sap from the puncture" of such feeding specimens, or "the trunk and larger limbs quite wet with the sap which had escaped in this way," as described by Quaintance. These out-door studies were often made in the early hours of the morning when dew was dripping from every bush but it was not supposed that this was in any case an exuded sap of the plant tissues upon which the insects were found! But neither under these field observations nor in those carefully restricted to the inclosed insectaries already referred to, was the writer able, except in the rarest instances, to convince himself that feeding occurs at all; certainly it is not a *common and general feature of adult life*. Furthermore, numerous dissections made of the insects have failed to show the gorged conditions of stomach and rectum which is described by several as plethoric to the point of rupture on the slightest disturbance!

Feeding habits among other animals of similar life cycles confirms what has just been pointed out. It is well known that many other insects have similarly anomalous habits and life

histories, and that among them are various species of ephemerids. From the early accounts of these insects so graphically described by Swammerdam ("Natural History of Insects," Eng. trans., 1758, pp. 103–27), on to the present time, it has been common knowledge that these and other species live as larvæ for many months or even two or three years an aquatic life. During this time, they are voracious feeders. Finally, at the time of metamorphosis, they emerge in enormous swarms during the summer, chiefly at evening, having relatively few hours of adult life, during which mating takes place and soon after the discharge of eggs, the early death of the adults. During this brief period of adult life, they take no food; the digestive system, and especially the mouth organs, being so imperfect as to render them incapable of active function. But like the cicada, these insects have the body tissue loaded abundantly with fat, which, in view of the extremely brief period of activity, can hardly be needed for nutritive purposes, but is doubtless utilized in the main for the rapid growth and perfecting of the generative organs and their products. I have verified these observations repeatedly and I am quite able to confirm what is more formally stated by Metchnikoff ("The Nature of Man," pp. 271–277). He shows that the rapid death following the act of mating and the discharge of eggs cannot be attributable to this act in itself since many males which have not undergone this action owing to the great excess of male insects yet die as promptly as do others which have participated in the process. He also shows that death cannot be due to the presence of pathogenic organisms since diligent search has failed to reveal their presence; and further that it is not due to phagocytic action, since the organs show no indication whatever of such invasion. He suggests the probability that such rapid death may be the effect of the early death of the cells of the nervous system, yet gives no evidence in support of the suggestion. In a later work, "The Prolongation of Life," this author emphasizes the significance of natural death in many groups of lower animals and the unique modes of providing against its hazards to the continuity of the species. Among these, he cites observations and experiments upon Rotifera (pp. 113, 118). "The whole course of life from the laying of the eggs until death lasts only about three days and is probably the shortest duration

of life in the animal kingdom. . . . The little males begin to swim soon after hatching, the wheel apparatus and the musculature being vigorous. They seek out the females, as their reproductive organs are mature at the moment of hatching. The transparent body, which is devoid of digestive apparatus, swarms with mobile spermatozoa. As soon as the male has seized the female, he discharges the contents of his body. It might be supposed that such an evacuation would cause violent perturbation of the system leading to the death of the organism. But the males are able to live for many hours after having accomplished their function, and the period represents a third of their natural duration of life. Moreover, I have isolated males from the females without any prolongation of their life. In one experiment, I isolated two males and placed a third in company with two females. It was the third specimen that lived longest. There can be no doubt but that the death of these male rotifers is natural in the fullest sense. The females, although they are provided with complete digestive organs, do not escape a similar fate. In some Ephemeridæ, which supply good cases of natural death, the end comes after a few hours of adult life without any sign of degeneration of the organs. As in others (Chlöe) life lasts several days without food ever being taken, it is clear that inanition is not the cause of the swift arrival of death in the first set."

As will be perceived, these citations from Metchnikoff relate primarily to distinctly different problems. But they are not without a measure of significance in connection with those under review. One point of importance is the fact that in these widely differing groups of organisms certain very fundamental functions, especially that of reproduction, take place normally during a period of inanition. Granting that the phenomena related of the Rotifers may be somewhat exceptional, and of only incidental significance, certainly those exhibited by the Ephemerids are clearly significant and pertinent, and have much in common with those so conspicuous in the life history of *Cicada;* thus accentuating the occurrence of kindred phenomena in widely differing organisms.

Fat Storage in Animal Economy.

The phenomenon of storage of fat among animals is a fact very well, and long known, and its physiological significance has been also generally recognized. Its occurrence in animals which pass long periods of hibernation, during which no food is taken, hence are dependent upon those reserve sources for sustenance, is a matter equally well known and common in many groups of animals. Among these are mammals, reptiles, amphibia and fishes. In the last group are cases in which such reserves are accumulated to meet extra and unusual demands which are involved in extended migrations to distant spawning grounds. And further dependence upon this store of energy is required for the maturation and fertilization processes involved in the reproductive crises common to many of this class of animals. This phenomenon is known in numerous species among which the salmon is a familiar example, with the rather tragic consequence that this climactic performance is usually followed by immediate or early death of the organisms.

Some recent investigations and experiments of rather striking importance have been made by Prof. C. W. Greene concerning the physiological processes, both of the storage of this reserve material and its later resorption by the tissues. (Bull. U. S. Bureau of Fisheries, 1914, pp. 73–138.) Professor Greene has studied this especially in the King salmon during the long fast of the spawning migration. He shows how the storage takes place in the musculature and connective tissues during the late growth, and especially the voracious feeding just prior to the migratory ordeal which involves hundreds of miles up great rivers and against many and serious obstructions. The energy consumed during this ordeal must be supplied by these reserve sources of nutrition. And as just pointed out, the additional demand involved the growth and ripening of the sexual products and actual spawning of these at the end. It will be at once perceived that for such an ordeal very large resources of energy must be available and these are to be found almost wholly in these reserve fat materials. Many other such experiments have been made upon various species of animals such as amphibia, reptiles, etc., all going to sustain the above cited findings;

namely, that the storage of potential energy in the form of fats or surplus proteids is an obvious provision for maintenance of vital functions during periods of reduced or suspended activity, but which is made available by a reversed metabolism, brought about by the operation of identical or similar enzymes, as shown below. Greene has, by actual experiments and extended observations during these migrations of the salmon, shown with clearness and convincing results the entire physiological history of the absorption and storage of fats, and its later transportation to the various tissues and organs concerned. He also critically reviews the earlier work of Miescher along these lines and emphasizes its values, at the same time showing certain of its defects, especially its erroneous contention that the fats found in muscular tissues were degeneration products; and shows convincingly that the presence of fat in such tissues is a result of infiltration and "that intracellular fat of the King salmon is an expression of the nutritive state of the muscle. It is a loading of fat by a process of infiltration and is not a degeneration of the muscle substance." He next points out the applicability of the same discovery by Kastle and Lovenhart of the reversible action of lipase and, as a consequence, gets an insight into the mode of transportation of fats from tissues to tissues in the animal body. (*Ibid.*, pp. 123–125.) These researches of Greene throw fresh light upon very similar problems of reserve energy of storage fats well known in invertebrates. For example, the cases of the Ephemerids, Lepidoptera, and the periodical cicada, all of which show points of close similarity to the foregoing. Among insects, this reserve material is accumulated chiefly in a peculiar organ known as the "fat body" which is "of various shapes," according to Packard, "more or less lobulated and net-like and covers parts of the viscera, also forming a layer under the integumen. The tracheal endings are usually enveloped by the fat body. It is larger in the larvae than in the adults, especially in Lepidoptera, in them forming a reserve nutrition used during the metamorphosis and during the formation and ripening of the eggs and male cells." (p. 419.) According to Wheeler whom Packard quotes, this fat body is of mesodermal origin, and differentiated from portions of the cœlomic walls, hence of metameric origin. Numerous more or less conflicting accounts have been given as to the partic-

ular function of this body. For example, Marshal regarded it as a urinary organ; Graber regarded the entire system of the fat bodies as a simple many-lobed lung; a view likewise taken by Landois; but Schäffer took the view, now generally held, that it is a reservoir of nutrition from which the organism may draw during times of special stress or emergency. The case of the cicada is peculiar; for its whole larval and pupal existence comprises from thirteen to seventeen years of underground life devoted especially to feeding and growth. These finally culminate in the crisis of reproduction which lasts only two or three brief weeks. But during these weeks, feeding is almost wholly lacking as has already been previously shown. When first emerging into adulthood the body of this insect is literally gorged with storage fat and related reserves. But these rapidly decrease with growth and development of the reproductive cells, and with the maturation and discharge of these cells, this reserve supply becomes rapidly exhausted, especially in the male, and the female completes its exhaustion in the arduous task involved in puncturing branches and twigs for receptacles in which the eggs are laboriously deposited. These functions completed, the vigor of the insects rapidly declines, since the storage being exhausted and taking no new supplies, they rapidly decline and die. As an interesting incident bearing upon the matter, may be stated the fact that in the use of these insects as an article of food, which is common among American Indians, they are taken exclusively at the time the insect emerges in its mature form, or at least, very soon after, for at this time this storage matter is at its best and later, of course, rapidly deteriorates. At this period, also, the insects are preyed upon by hogs, fowls, and such birds as feed upon them, since only at this time are the insects easily available. For birds, they can be taken during the entire life period of a few weeks, but naturally, are most sought in early life when they are more easily captured.

There are many analogous features between the physiology of fat storage as shown in the foregoing citations from Greene's experiments and what probably takes place during the life cycle of these insects, some before mentioned. Among others the following are of interest.

1. The relatively long and probably more or less continuous

periods of feeding and growth. Experiments show that for the salmon it may be five to eight years; for the ephemerids two or three years; while for the cicada thirteen and seventeen years. In these groups this long period is now believed to be generally concerned in accumulating reserve potential energy, most of which will sustain an important relation to the brief, but crucial period of activity and perfecting the reproductive elements, and their union for the preservation of the species.

2. In each of these groups this actively cumulative growth and storage of energy, followed by a relatively brief period of reproductivity, gives that anomalous reaction of decline and death a unique significance.

3. Corresponding to these extended and painstaking researches of Greene I know of nothing among insects or other invertebrates; but the remarkably analogous aspects of the cases lead me to conclude that the physiologic activities involved in the latter are more or less similar; in some respects identical, with the former.

The Posterior Crop.

Under this caption, the junior author in his earlier paper has described a most unique and anomalous organ, clearly, as I believe, a part of the digestive system. For a full account of its anatomy reference may be made to his description in the paper just cited. It must suffice here to briefly summarize its main features. During nymphal life, as is well described in the second section of this contribution, it is rather small, "with walls of uniform texture and much folded. But in the adult, the walls of the organ are distinctly variable in thickness. The outer surface of the organ is closely apposed on all sides by fat. This probably has something to do with the collapsed condition of the tube in this region." A recent popular paper by Snodgrass descriptive of this insect designates this organ on the contrary as a part of the respiratory system. But, as will be shown later, this seems decidedly erroneous. In my earlier account (Proc. Onon. Acad. Sci., 1903, p. 51) I conceived it in adult life to act in some way as an organ for aiding in the absorption of fat, its epithelium in many cases being more or less charged with globules of fat. This was confirmed by the work of Henderson who also

showed that in no case of his numerous dissections, more than two hundred in all, of either nymph or adults, did he find traces in this organ of undigested food.

All this is abundantly confirmed by my own later work as it in turn also confirms earlier observations as to the feeding habits of these insects. Likewise, this is found borne out by the microscopic sections of the canal through every region of the mature insect by the junior author as shown in his previous paper. But there are certain rather puzzling features in this particular organ in adult life. It does not appear clear that its increase in size at this time can be due to a reservoir function, unless feeding be increasingly active during later life, which certainly does not seem to be the case. Again such a view seems to be in direct conflict with the histological character of the organ which shows clear evidence of degenerative changes in its lining epithelium. On the other hand it seems to conform with the view just previously expressed that it is during this stage apparently functionless in any active way, and that as the storage elements are resorbed the organ reacts in consequence, its walls expand to occupy the visceral spaces which earlier were filled by storage matter and reproductive organs, which in turn accounts for the attenuated condition of the epithelium described by Hickernell as above stated.

A further fact in this connection remains to be noted, namely, that these adult insects leave no signs of excretory wastes, such as defecative products. The writer has handled living specimens by hundreds, taken at various times, some kept under bell jars, others in clean breeding cages, as well as others still which were freely handled, but has not seen at any time evidence of defecation. Of course, the liquid sap upon which these insects feed might, and doubtless does show less of solid waste to be discharged, but certainly there are unused elements even in such foods as sap which doubtless are extruded; as are those heavier products whose wastes are so conspicuous in the life surroundings of most feeding insects. The well-defined and thick-walled rectum of the cicada goes to support this view. This point is made as a matter of fact which should not be overlooked; but so far as I am aware it has not been given attention heretofore. Were feeding at all frequent or general in the adults such solid

excreta could hardly fail of notice. Their absence, therefore, can hardly be other than highly significant of the lack of active digestive operations during adult life, and is in entire accord with the fact of the entire absence of alimentary elements in the tract as above cited.

During the progress of this work my attention was called to a popular paper by R. E. Snodgrass on the Seventeen Year Locust (Smiths. Rept. for 1919, Washington, 1921, pp. 381–409), in which there appear certain views rather sharply in conflict with those herein maintained, and which call for some brief attention. Its anatomical points are reviewed by the junior author in the section which follows. But it falls to me to notice phases of feeding habits and others of a physiologic nature. In reply to certain inquiries submitted to Snodgrass he was kind enough to write me quite freely as to the questions, and also sent a specimen of transected insect to show the highly cavernous aspects of late life, and to afford what was suggested as a demonstration of the tracheal nature of the so-called "air-chamber." This, I examined with care, but cannot accept as demonstrative, since there were no distinct evidences of its tracheal structure, as the junior author conclusively shows in the histologic demonstrations of all phases of the typical and deteriorative epithelial cells occurring, and an entire absence of chitinous elements or tænidia in the organ.

Of this "airchamber" Snodgrass states that it "receives its supply of air directly through the spiracles of the first abdominal segment." If this were so there should be unmistakable evidence of its being a paired organ, as in the bee; but of this there is no evidence whatsoever, a fact which he admits but claims such to be the case in the dog-day cicada. This I have not been able to discover from actual dissections, or from serial sections of the regions, as shown by Hickernell in the earlier paper.

Referring to its function as related to the respiratory system Snodgrass without hesitation discredits the view of Graber, that it may have some relation to the tympanal organs, but says, "We shall probably have to fall back on the old prosaic explanation that bulk of body is maintained with corresponding weight eliminated—a combination specially favorable to aërial life." But as Packard long ago pointed out (cf. p. 457), this assumption

is erroneous, "The body of the insect during flight not being lightened by the air in the sacs." Submitting this point to two of my colleagues of the department of physics, Doctors Porter and Packard, I am assured that it is entirely correct.

It seems rather certain, therefore, that neither from its structure, nor yet from the "old prosaic explanation" of an adaptation to aërial life, does he sustain his conclusions. Granting the ramifications of tracheæ over the organ no more makes it respiratory than does a similar disposition of tracheæ over the viscera or the musculature constitute them such. Apart from the names by which Graber designates the organ, namely, "Tracheenbläse" and "Luftsäcke," there is nothing in his account which supports the views of Snodgrass. Graber's problem was the "Tympanalorgane," not respiration. I believe therefore that Snodgrass has misconstrued Graber's work so far as it is applicable to the matter at issue, and that his view may be dismissed as devoid of structural or functional evidence as a respiratory organ, or as an accessory "Tympanal organ," as claimed by Graber (p. 282–3).

LITERATURE CITED.

Graber, Vitus.

'77 Die Insecten, München.

'76 Die abdominalen Tympanalorgane der Cicaden u. Grylloden. Denkschr. d.k. Akad. d. wissen. Math., natur classe, Bd. 36, pp. 273–296.

Greene, C. W.

'14 Storage of Fat in the Muscular Tissue of the King Salmon and its Resorption During the Fast of the Spawning Migration. Bull. Bur. of Fisheries, Vol. 33, pp. 73–138.

'14 Fat Absorbing Function of the Alimentary Tract of the King Salmon. Ibid., Vol. 33, pp. 149–175.

Hargitt, C. W.

'03 The Periodic Cicada, Proc. Onondaga Acad. Sci., Vol. I., 1903, p. 41.

Hickernell, L. M.

'20 Digestive System of the Periodical Cicada. Ann. Ent. Soc. Am., Vol. XIII., pp. 223–242.

Marlatt, C. L.

'07 The Periodical Cicada. Bull. 71, U. S. Bur. Entomol.

Packard, A. S.

'98 A Text-Book of Entomology.

Quaintance, A. L.

'02 The Periodical Cicada and Its Occurrence in Maryland. Bull. No. 87, Maryland Agr. Exp. Sta.

Snodgrass, R. E.

'19 The Seventeen Year Locust. Smith. Report 1919, Pub. 1921, pp. 381–409.

THE DIGESTIVE SYSTEM OF THE PERIODICAL CICADA, TIBICEN SEPTENDECIM LINN. III. MORPHOLOGY OF THE SYSTEM IN THE NYMPH.

L. M. HICKERNELL,

SYRACUSE UNIVERSITY.

In an earlier paper, the form and relationships of the various parts of the digestive system of the adult cicada were considered. It was found that in both sexes the system is complete and well organized but much complicated by a winding and twisting together of its parts, so that the continuity of the system could be established only with some difficulty. The intertwining intestine, esophagus, and malphigian tubules in the anterior part of the body cavity makes the "internal gland" or filter which seems to be characteristic of so many of the Homoptera. This structure, while different in some respects in the cicada from that described in other homopterous forms, has a general arrangement which is similar.

It was found further that certain organs of the digestive system change shape markedly, as the insect increases in age, but that the ground plan of the alimentary tract remains the same throughout the life of the insect and that the system does not degenerate or become broken as has been believed by certain workers.

Because of the numerous peculiarities which were found in the adult digestive organs, it seemed worth while to examine the nymphal stages with the object of determining what conditions exist there and also to determine, if possible, the origin of some of the peculiar structural relationships. For, although the peculiarities of the homopteran digestive tube have been recognized for some time, the beginnings of these peculiarities in embryological history have not been figured or explained in many cases. It can at least be established whether the peculiar windings of the digestive organs arise at the time of one of the numerous moults or are to be traced back to the egg itself.

The consideration of the morphology of the digestive organs of the Homoptera dates back to the work of Lubbock, Leydig,

and Ramdohr about the middle of the last century. Lubbock described the digestive organs in *Coccus hesperidum*. He observed the complicated windings in the anterior region and called this complex the internal gland.

Dufour (1833) described the digestive apparatus of certain Hemiptera but the significance of some of the structures which he described apparently escaped him. In fact, it has been only recently that the digestive systems of any great number of Hemiptera have been worked upon and the true nature of the structural peculiarities of the organs determined.

Witlaczil ('85) studied the anatomy of the Psyllidæ. In *Psyllopsos fraxinicola* the digestive canal has many of the peculiarities which have been noted in the cicada.

Berlese (1909) describes the digestive organs of certain scale insects. In these insects the rectum is large and extends anteriorly as far as the esophagus. This results in a knitting together of rectum and esophagus and also causes the intestine to describe a complete circuit of the abdominal cavity before it finally joins the rectum. This condition, while resembling it superficially, is entirely different from the arrangement found in the adult cicada since in the latter the enormous enlargement affects the mid-gut while the rectum is relatively small.

Licent (1911) gives an account of the digestive organs in the Cercopidæ. In this family there is also a loop made by the mid-gut which curves backward so as to become intimately associated with the anterior part of the canal. Licent believes with Berlese that this complication acts as a filter, allowing the watery part of the food to diffuse directly through the walls of the fore-gut into the cavity of the hind-gut and in this way saves the mid-gut for digestive activity upon the more concentrated food mass. The nutritive substances, greatly diluted with sap, are thus concentrated and, for the most part, digested in the mid-gut.

Kershaw (1913) recorded his observations on the digestive organs in *Siphanta acuta*, a flatid. This form has a large reservoir or crop, which from its junction with the esophagus just within the abdomen, extends anteriorly above the esophagus through the thorax and practically fills the epicranium above the brain. It also extends posteriorly beneath the heart and above the rest of the internal organs, almost to the tip of the abdomen. It is

interesting to note that this food reservoir is similar in most respects to the enlargement in the digestive tube of the cicada, and furthermore that it attains its enormous dimensions only in the later adult life of the insect. In this last respect it further resembles the condition in the cicada.

From the above accounts taken with other works not quoted here, it seems evident that the digestive systems of the related forms of the group under consideration studied to date have, in general, the same ground plan and that their specializations although varying in degree, are mostly of the same kind. These structural complications have led to different interpretations of the relationships and functions of the various organs, but it seems likely that there is a much closer similarity in these respects than at first appeared to be the case.

The conclusions here presented are based upon a study of nymphs of *Tibicen septendecim* Linn. of four different sizes, viz. 2½ mm.; 7 mm.; 14 mm.; and 21–24 mm. This series covers practically the entire period during which the insect lives under ground. The 2½ mm. nymphs are only recently hatched from the egg, while the 21–24 mm. individuals were full grown nymphs dug from the ground about one month previous to the general emergence and transformation of the brood. According to Marlatt, the nymphs of 7 mm. body length are about four years old, while the 14 mm. ones are about ten years of age. These relationships between size and age were established by workers in the Bureau of Entomology, who followed a complete life-cycle of one of the cicada broods.

The method of serial sectioning and reconstruction was followed throughout this study. The impossibility of tracing the alimentary canal by means of gross dissections is even more apparent in the immature forms than it is the adult.

In describing the digestive organs as found in cicada nymphs of different ages, it would be more logical to begin with the youngest and follow the developemental series until the adult condition is reached. However, in an earlier paper the adult digestive organs were described and the descriptions and comparisons here given have been written keeping the adult structure in mind. Obviously, in such a situation it will be easier for the writer and more understandable for the reader to work back-

wards through the series of nymphal stages where structural variations are relatively slight, as between two successive stages, than to jump from the adult condition back to the earliest nymph and then work up to the adult condition once more. It is believed that a description of these different stages in reverse order is warranted in the present instance in view of the circumstances outlined above.

The alimentary tract in nymphs of 21–24 mm. length has the same general arrangement of parts as is found in the adult. The form and structure of the individual organs varies, however, in some respects. The posterior crop (Fig. 1, *pc*) always has its walls wrinkled and contains a tortuous lumen, as if the structure had collapsed completely, while in the adult this division possesses smooth, thin walls surrounding an enormous lumen. The size relations of some of the other parts also vary as is shown hereafter. A general view of the entire system as seen from the left side is represented in Fig. 1. The esophagus is a simple tube, uniform in diameter, and leads into the anterior crop (*ac*). The latter with its dorsal adherent "internal gland," is of about the same relative size as it is in the adult. The anterior crop empties through a narrow opening into the posterior crop (*pc*). The latter is greatly unlike the corresponding division in the adult. Its relative length is much the same but its walls are uniform in texture and much folded throughout. In the adult the walls of the organ are distinctly variable in thickness and are not folded to any extent except at the extreme anterior end where the cæcal projection runs ventral to the anterior crop. The outer surface of the posterior crop is closely apposed on all sides by fat. This probably has something to do with the collapsed condition of the tube in this region. At any rate, its opposing walls almost touch each other throughout its whole extent, thereby making the lumen narrow and irregular (Fig. 4, *pc*).

The ascending intestine is relatively larger in diameter in the 21–24 mm. nymph than it is in the adult. Its general course and connections are the same as in the adult but its size, both externally and with respect to its lumen is noticeably greater (Figs. 1 and 4, *at*). The ascending intestine enters the internal gland in the same fashion as it does in the adult. The descending

intestine also emerges from the complex of tubes as it does in the fully developed insect.

The descending intestine is relatively smaller in this stage than it is in the adult condition. Emerging from the internal gland it runs in a general posterior direction as in the adult, finally making a knot or coil (Fig. 1, *kk*) just before emptying into the rectum. There are some variations in the histological structure of the epithelium in its walls but the tube is easily recognized in section when one is familiar with its microscopic structure in the adult.

The rectum does not differ greatly from that in the transformed insect. It receives the descending intestine and then gradually narrows until the anal opening is reached.

Among the younger nymphs there are variations in size and arrangement of the digestive organs but these are slight as compared with the structural differences shown between the organs of the early nymphs and those about to transform to the adult condition. In nymphs of 14 mm. and 7 mm. body-length there is not enough variation in the arrangement of the organs in the two stages to warrant separate description. Figure 2 is based upon specimens of 14 mm. length but, except for size, the same figure applies to the shorter and younger stage. Esophagus, anterior crop, and internal gland are practically the same as in the stage previously described. The remaining portions of the system, however, differ greatly in many respects.

The posterior crop does not have any suggestion of saccular structure in these early stages. Its walls are folded as in the other previously described specimens but it does not assume the enormous diameter common to that division in the older nymph and adult. In fact, the ascending intestine has a larger diameter in this stage than does the posterior crop. Its course is almost straight through the center of the body until it reaches a point just anterior to the rectum. Here it joins the ascending intestine.

The ascending intestine (Fig. 2, *at*) is exceedingly prominent at this time. It is much convoluted and extends posteriorly in the region of the rectum, from which place it runs anteriorly. For the most part it runs along the ventral surface of the body and when it reaches the internal gland it disappears as in the adult. The interweaving of the posterior crop and the two intestinal divisions seems difficult of interpretation when first

studied in sections. However, the general ground plan is soon seen to be in no way different from that in the stages previously described.

The internal gland has the same structure here as it does in the older nymph. In size, it is of course, smaller but sections show identical parts in the two stages.

The descending intestine and rectum have the same form and arrangement in the 7 mm. and 14 mm. nymphs as they do in the later stages. The diameter of the descending intestine is very small. It runs close to the dorsal integument in some places and might easily be missed in studying sections. The characteristic coil (Fig. 2, *kk*) exhibits a convolution which is practically the same as that described for the later stage.

Examination of Figs. 1 and 2 makes it clear that there is no great difference in the arrangement of the digestive organs of the nymphal stages considered. It is also true that in the $2\frac{1}{2}$ mm. nymph there is not enough difference in structure or arrangement to warrant making a separate figure to represent conditions there. This means that the plan of the digestive apparatus is not altered practically throughout the entire underground existence of the insect. The variation in the size of certain organs at different periods in the life-history suggests either that the nymph does not feed continuously or that there is a change in the function of some of the organs as time goes on. The former supposition is probably correct for it is known that these immature forms have alternate periods of feeding and resting.

The ascending intestine is found in some sections to have its epithelial lining made up of enormous cells filled with granules. In other cases we have the condition represented in Fig. 4, *at*, where the walls of this organ are thin and attenuated. These variations probably represent different phases of functional activity and are not to be interpreted, therefore, as indicating any change in the plan of digestive activity.

The complication of organs in the anterior region which has been called the internal gland, arises at a time earlier than that represented in the stages here described. Fig. 3, which is a transverse section through the internal gland region of a 24 mm. nymph, shows all the parts arranged in a manner similar to that in the adult. In Fig. 5, which is a like section through the same

region in the 14 mm. nymph, the same organs are found as before, but there is slightly less complication in the way of folding and intertwining than is found in the later stages. Figures 7 and 8 represent transverse sections through the anterior region of the digestive organs of a 2½ mm. nymph. Here again the various organs are seen to have assumed a position similar to that in which they are found in the later nymphs and adult. The peculiar relation of anterior crop, intestine, and malphigian tubules is, therefore, established in all these nymph stages the same as in the adult. In seeking the origin and significance of this arrangement, it is necessary, then, to go back to the development of the embryo within the egg.

In Figs. 1 and 2 the malphigian tubules have not been represented. They are present in the same number and arrangement as in the adult. They have been left out of the above figures since they only tend to obscure the clear representation of the digestive organs.

The function of the posterior crop as an accessory storage organ seems, at first, to be indicated by a comparison of the different stages here considered. Originally a tube of small diameter, it increases in size until it exceeds any of the other organs in capacity. I have never found any precipitate or coagulum in the cavity of this organ, however, so that the mere size of its lumen may not justify one in attributing a storage function to it.

In summarizing it may be said that from the observations upon the four nymphal instars of the cicada it is evident that the digestive organs show an arrangement which is similar in ground plan with that of the adult and also it is similar in many respects with that of other Homoptera which have been described. The complication of digestive organs in the anterior region of the insect is fully established in the earliest nymph and hence is developed at a time previous to that represented in the material here considered. The posterior crop loses its simple tubular character and becomes saccular at some time after the 14 mm. nymph stage.

Since the foregoing part of this paper was written, the paper by Snodgrass dealing with the anatomy of the cicada has come to hand. In this publication the large vesicular organ which

occupies the greater part of the abdominal cavity in the adult insect and which I have called the posterior crop, is considered as a part of the respiratory system. The chief reason for this is the apparent continuity existing between this sac and the first pair of abdominal spiracles. Mr. Snodgrass has been kind enough to demonstrate dissections to me which seem to bear out his contentions. There are, however, some fundamental objections to his position.

In the first place, a "tracheal bladder" or respiratory duct of any kind in an insect should show a lining layer of chitin since the tracheal system of insects arises as an invagination of the primitive ectoderm. In a former paper sections of the "tracheal bladder" of Snodgrass through three different regions were shown and none of these showed any evidence of a chitinous layer. It has been suggested that perhaps this structure attained its respiratory function secondarily and hence might not conform in all structural details to expectations. It is hard to imagine how an entire segment or organ of the digestive tube could undergo such a transformation of function.

In my sections also, I have shown that there are distinct openings at the anterior and posterior ends of this organ, the one at the posterior end leading into a continuation of the digestive tube and that a muscular valve intervened between these two divisions. In view of this evidence it is difficult to conceive of this part of the abdominal contents as having a respiratory function.

It is easy to be deceived as to the continuity of the lumen of the posterior crop with the exterior through these first, abdominal spiracles. In gross dissections there is only the most delicate epithelial membrane limiting this abdominal sac in the region of these spiracles. In my earlier paper a figure was shown covering this point. If a specimen is allowed to become dry, the portion of the wall which is in front of the spiracular opening may easily rupture and then there is an external opening in fact.

Sections show that the spiracle opens into a very small chamber the walls of which break up almost immediately into a number of tracheal tubes which distribute themselves *over the external surface* of the posterior crop. I am therefore, still inclined to question any interpretation which gives this organ a respiratory

function. It certainly becomes modified in later life but it is at all times a part of the digestive system. This condition which is easily observed in sections makes unnecessary the postulation of any secondarily derived function on the part of this organ. The method of gross dissection, then, is inadequate to explain the conditions found.

BIBLIOGRAPHY.

Berlese, A.
'09 Gli Insetti.
Dufour, L.
'33 Rech. anat. et physiol. des Hemipteres.
Hickernell, L. M.
'20 Ann. Ent. Soc. Am., Vol. XIII., No. 2.
Kershaw, J. C.
'13 The Alimentary Canal of *Flata* and other Homoptera. Psyche, Vol. XX., No. 6.
Licent, E.
'11 Signification de la dilation proventriculaire chez les Homopteres superieure (Hem.). Bull. de la Soc. Ent. France.
Marlatt, C. L.
'98 U. S. Dept. Ag. Div. Ent. Bull., 14, N. S. U. S. Dept. Ag. Bur. Ent. Bull., 71.
Snodgrass, R. E.
'21 Smithsonian Report for 1919, pp. 381–409.

EXPLANATION OF PLATES.

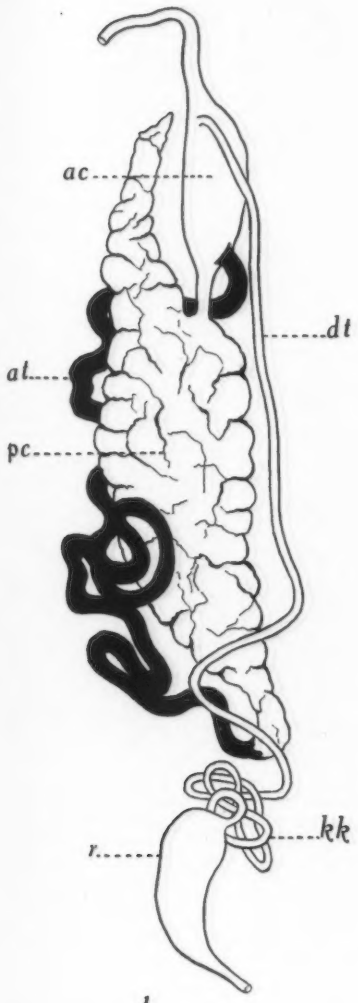
INDEX TO FIGURES.

ac—anterior crop.
at—ascending intestine.
dt—descending intestine.
int—internal gland.
kk—coil in descending intestine.
mt—malpighian vessels.
pc—posterior crop.
r—rectum.

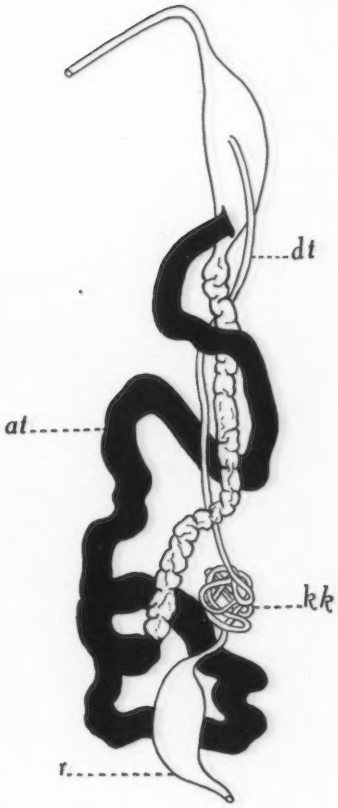
PLATE I.

FIG. 1. Digestive system of cicada nymph of 2 mm. body length, seen from the left side.

FIG. 2. Digestive system of cicada nymph of 14 mm. body length, seen from the left side.



1



2

L. M. HICKERNELL.

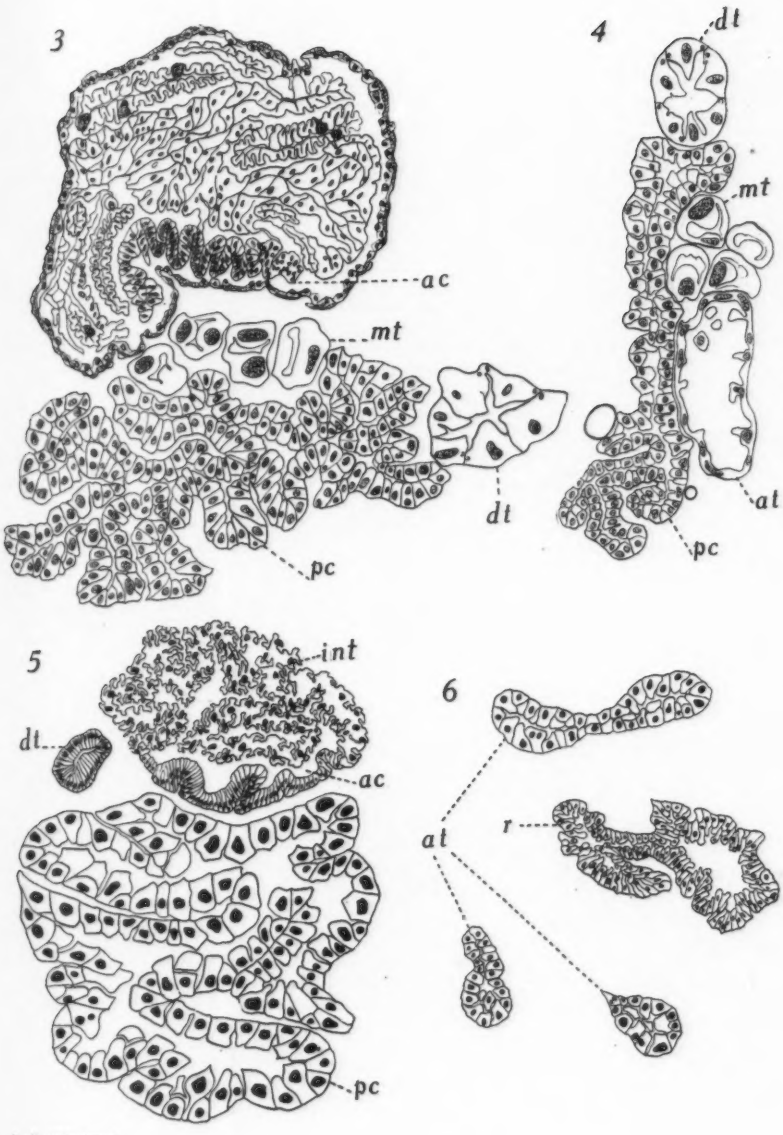
PLATE II.

FIG. 3. Transverse section through the internal gland region of a 2 mm. nymph.

FIG. 4. Transverse section through digestive organs in anterior region of a 7 mm. nymph.

FIG. 5. Transverse section through internal gland region of a 14 mm. nymph

FIG. 6. Transverse section through digestive organs in posterior part of a 7 mm. nymph.



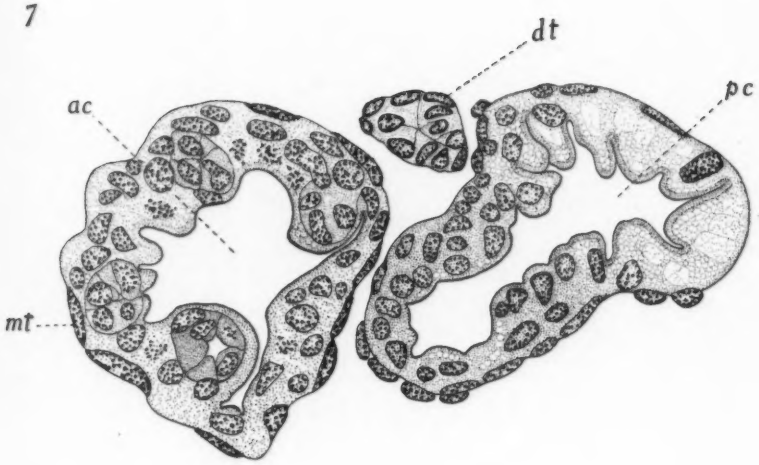
L. M. HICKERNELL.

PLATE III.

FIG. 7. Transverse section through anterior part of digestive organs of a nymph recently hatched.

FIG. 8. Transverse section through same region but slightly anterior to that represented in Fig. 7.

7



8

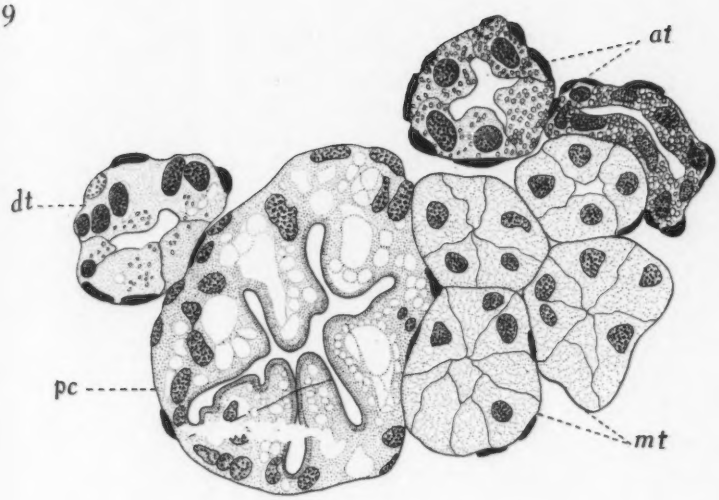
L. M. HICKERNELL.

PLATE IV.

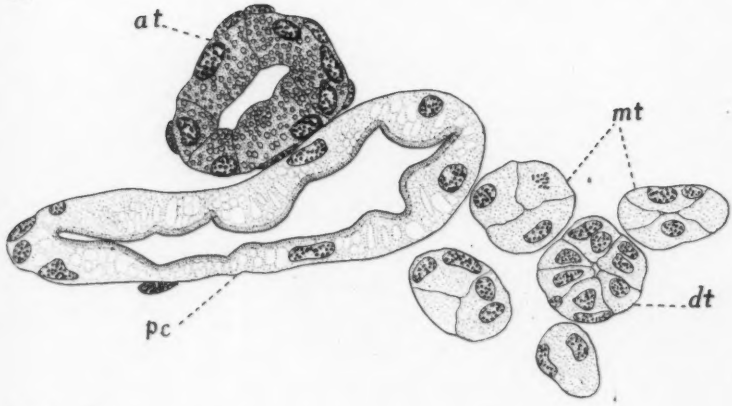
FIG. 9. Transverse section through digestive organs in mid-body region of a nymph just hatched.

FIG. 10. Transverse section through posterior part of digestive organs from the same series as Fig. 9.

9



10



L. M. HICKERNELL.